ON SUGAR IN THE URINE.

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ON SUGAR

IN THE URINE,

By H. Bence Jones, M.D., F.R.S.

Part 1. *On the detection of Sugar when added to Healthy Urine.*

The detection of small quantities of sugar in water and in a solution containing organic and inorganic substances, constitute two questions as different as the detection of small quantities of arsenic or opium when dissolved in pure water or in a compound fluid.

Nothing is easier than to determine the presence of small quantities of sugar, arsenic, or opium in distilled water; but when organic matters are also present, the difficulty of the analysis becomes sometimes excessive. Very small quantities cannot be detected. The limit that can be found varies with each substance, according to the nature and amount of the organic matter present, according to the process of separation used, and according to the skill of the chemist.

In cases of poisoning, the separation of the poison from the contents of the stomach, or from the substance of the different

organs of the body, constitutes the whole difficulty; and this is also true regarding the detection of sugar. Whatever process is used for separating the sugar and the organic matter, some difficulties will be met with, and some limit to the quantity of sugar that can be detected will be found.

It is the object of the first part of this paper to show the difficulties and limits which exist when Lehmann's process for detecting sugar in the urine, or the fermentation process, or Soleil's Saccharimeter, or Brücke's processes are used.

I am much indebted to Drs. Ulrich and Valentine for carrying out my wishes, and for making the results as trustworthy as possible.

Lehmann's Process.

In his "Physiological Chemistry" (Translation, p. 285, vol. i), Lehmann says, "If a specimen of urine contain very little sugar, it is advisable to extract the solid residue with alcohol, to precipitate the sugar by the alcoholic solution of potassa, to dissolve the compound of sugar and potassa in water, and then to apply the sulphate of copper test."

Some experiments were first made on the solubility of potass-sugar in alcohol of different strengths.

200 c. c. of alcohol were made of three different degrees of strength, 90°, 80°, 70°, and the same amount of grape-sugar was added to each. The rotation was found, by the saccharimeter, to be between 8° and 9°; the temperature was 18 C. (64° F.) The sugar was precipitated by potassa, washed with absolute alcohol, and dissolved in water; and the solutions were neutralised with hydrochloric acid, evaporated, decolorised, and examined by the saccharimeter. The alcohol of 90° then gave 4° of rotation. The alcohol of 80° gave 2° of rotation. The alcohol of 70° gave no rotation.

A similar experiment was repeated with an amount of sugar which gave 11° to 12° rotation. The alcohol of 90° gave 6° of rotation. The alcohol of 80° gave $4\frac{1}{2}$°.

1 grn. of pure grape-sugar was dissolved in 50 c. c., in 100 c. c., and in 150 c. c., of an alcohol between 80° and 90°, and potassa dissolved in alcohol of the same strength was added to each solution. In each a precipitate fell, but, after standing many hours, it was scarcely perceptible at the bottom of each glass.

1 grn. of sugar in 300 c. c. of alcohol between 80° and 90°, gave a scarcely perceptible precipitate with potass-alcohol.

On the other hand, the whole of the sugar was precipitated when 6 grns. of sugar were dissolved in 200 c. c. of methylated alcohol of 99°; and when 3 grns. were dissolved in 150 c. c. of alcohol of 98° to 100° treated with potassa, and the sugar-potassa determined by the saccharimeter, the whole was regained.

These experiments show that the evaporation of the urine must be carried nearly to dryness in order that the residue may be treated with nearly absolute alcohol. It was therefore necessary to determine the effect of the evaporation on the sugar added to the urine. Known quantities of sugar were added to urine before and after evaporation.

1000 c. c. of urine were evaporated; 6 grns. of sugar were then added to the residue, which was extracted with alcohol 98°, and ultimately 3 grns. of sugar were recovered.

325 c. c. of urine, treated the same way with 8 grns. of sugar gave 5½ grns. A second experiment gave the same result.

If to 100 c. c. of urine 8 grns. of sugar were added, and the evaporation in the water-bath was carried to dryness; 5 grns. were recovered by extraction with alcohol.

A second experiment gave the same result.

When the same quantity of sugar was added to 500 c. c., 4 grns. were recovered.

If to 500 c. c. urine 15 grns. were added, between 7 and 8 were recovered after evaporation.

In 1000 c. c., when 8 grns. of sugar were added, only 2 grns. were recovered. When the same quantity was added to 2000 c. c., a trace only was detectable.

Hence, during evaporation of small quantities of urine, there is but little decomposition of the sugar that is added; but when large quantities of urine are evaporated in the water-bath, much sugar is lost; and Lehmann's process for detecting small quantities of sugar in the urine is not sufficiently delicate.

On the Fermentation Test.

Of all the tests for sugar, the most conclusive, when it can be obtained, is the production of carbonic acid and alcohol by yeast.

The following points were examined:—1. Whether equal quantities of yeast give off equal quantities of carbonic acid,—that is, whether any error arises from deducting the quantity of carbonic

acid given off by the yeast itself from the quantity given off by the yeast and sugar together? 2. What is the least quantity of sugar in water and urine that can be detected? 3. What effect urea, oxalate of urea, and the residue of the urine have on the process of fermentation? 4. What is the delicacy of the test as compared with the saccharimeter and Fehling's solution?

1. On the carbonic acid given off by yeast washed once with water:—

324·28 grns. of yeast gave 1·57 carbonic acid = 0·42 grns. per cent.
421·50 „ „ 1·79 „ = 0·48 „

In second experiment,—

240·70 grns. of yeast gave 0·75 carbonic acid = 0·31 per cent.
321·47 „ „ 1·00 „ = 0·31 „

2. What is the least quantity of sugar in water and urine that can be determined?

Half a grn. of grape-sugar with 29·99 grns. yeast, gave 0·39 grn. carbonic acid.

33·08 grns. yeast without any sugar being added gave 0·16 grn. carbonic acid.

Therefore 29·99 grns. yeast gave 0·14 grn. carbonic acid.

Hence, half a grn. of sugar gave 0·39 − 0·145 grn. carbonic acid = 0·24.

Theoretically = 0·24.

In a second experiment, after the yeast had been well washed to remove every trace of alcohol, the following numbers were obtained:—

Half a grn. of sugar, with 40·58 grns. of yeast, gave 0·34 carbonic acid.

Without sugar, 52·27 grns. of yeast gave 0·06 ∴ 40·58, give ·046.

Hence, half a grn. of sugar gave 0·34 − 0·05 = 0·29 carbonic acid.

The residue, after fermentation, was put into the smallest possible retort, and heated to boiling. The first drops that came over were tested for alcohol thus:—To one cubic centimetre of a moderately strong solution of bichromate of potassa, two or three drops of concentrated sulphuric acid were added, and then a few drops of the liquid supposed to contain alcohol. Heat was then gently used, and the fluid immediately became green from the alcohol present.

Hence, half a grn. of sugar in water gives carbonic acid that can be weighed and alcohol that can be detected.

One grn. of sugar was then added to 6 cubic centimetres of urine, and this fluid, without concentration, was fermented, and the sugar was detected. But if 1 grn. of sugar was added to 50 c. c. of urine, and this evaporated to 5 or 6 c. c., no fermentation occurred on the addition of yeast; neither was any fermentation observed when two or three grains of sugar were added.

If 3 grns. of sugar were added to 30 c. c. of urine, and this evaporated to 20 c. c,, the fluid quickly fermented, and the loss of weight was nearly the theoretical quantity.

The effect of urea and of oxalate of urea* was then determined.

The same sugar-solution was fermented without urea and with a small quantity of urea.

	Without Urea.		With Urea.
	1st Exp.	2nd Exp.	
Apparatus with sugar solution and yeast	1493·10	1506·63	1402·60
„ without „ „ „	1340·30	1351·60	1286·48
Yeast	152·80	155·03	116·12
Apparatus before fermentation . .	1493·10	1606·63	1402·60
„ after „ „	1472·26	1485·73	1381·64
Carbonic acid given off	20·84	20·90	20·96

Hence, the presence of the urea did not affect the fermentation.

If a concentrated solution of urea was taken, and 3 grns. of sugar were added to 6 c. c. of solution, no trace of fermentation occurred.

When 4 grns. of oxalate of urea were added to 34·6 grns. of sugar in solution, fermentation proceeded; but if much oxalate of urea was present, fermentation was stopped.

	4 grns. of Oxalate of Urea.	Much oxalate.
Apparatus with oxalate of urea and yeast	1618·90	1416·23
„ without „ „ „ .	1400·62	1295·08
Yeast	218·28	121·15
Apparatus before fermentation . .	1618·90	1416·23
„ after „ . .	1603·76	1415·20
Carbonic acid given off	14·94	1·03
Carbonic acid given off by yeast alone .		1·00

* Experiments were made with oxalate of urea, because this substance existed in the fermenting fluid when the urea was chiefly removed by oxalic acid added to the concentrated urine.

Hence, with much oxalate, the fermentation was almost entirely stopped.

On the comparison of the delicacy of the Fermentation-test, and with Fehling's standard solution.

A watery solution of grape sugar was prepared which gave a solution of 104°=2·288 grains of sugar in each cubic centimetre.* 15 c. c. of this solution=34·25 grains of sugar were fermented with yeast that had been once washed. A second experiment was made with the same quantities, and a third experiment was made to determine the carbonic acid in the yeast :—

	1st Experiment.	2nd Experiment.	3rd Experiment.
Yeast employed .	263·90	270·66	210·15
Carbonic acid given off	18·29	18·70	0·62
Carbonic acid in yeast	0·76	0·78	
Carbonic acid in sugar	17·53	17·92	

Hence sugar in each c.c. of solution =2·376 grains=2·437 grains

10 c. c. of the same solution were diluted to 160 c.c., and tested by fresh prepared Fehling's solution.

10 c. c. of Fehling's solution were reduced by 5·6 c. c. of the diluted sugar solution.

Hence 10 c. c. of the solution before dilution contained 21·9 grains sugar, and each c. c. contains 2·192 grains sugar.

Hence in each cubic centimeter of the solution there were present :—

By fermentation, 2·371 and 2·437 grs. sugar mean	=2·356 grs.
By saccharimeter	2·288 grs.
By copper test	2·192 grs.

On Soleil's Saccharimeter.

Before the saccharimeter can be used, the fluid about to be examined must be decolorised. To effect this, animal charcoal, acetate or subacetate of lead and ammonia, or chlorine gas must be used. These substances, whilst removing the colour, keep back or destroy some of the sugar, and it was desirable therefore to determine the loss.

* This number is obtained by making a solution of sugar which contains .01 gramme of sugar in each cubic centimetre when examined by Fehling's solution. This amount of sugar in solution gives seven degrees of rotation.

First, charcoal:—A colourless solution of sugar which gave 7° of rotation was mixed with animal charcoal, boiled for a few minutes, and left for a few minutes longer, before it was filtered. The charcoal was washed three or four times with hot water. The washings were added to the fluid which first came through, and the whole was then found to give the same rotation as at first.

A fluid, dark brown from the colouring matter of the urine had three grains of sugar added to it, and it was then mixed with animal charcoal; after standing some time, it was filtered, and as it was not colourless, it was again treated with animal charcoal, and this was repeated a third time. The animal charcoal was many times washed with hot water. The clear fluid ultimately obtained was examined by the saccharimeter, and the loss was found to be not more than the frequent filtrations might account for.

A colourless solution of sugar in water gave between 9 and 10° of rotation. 75 c. c. of this solution were shaken with a small quantity of purified and fresh-burnt animal-charcoal, the fluid was then filtered, and the charcoal was not washed. The solution then gave between 7° and 8° of rotation.

75 c. c. of the same solution with twice the quantity of animal charcoal gave a rotation between 5° and 6°.

75 c. c. of the same solution with three times the quantity of animal charcoal, gave a rotation of between 4° and 5°.

One volume and a half of the same sugar-solution was mixed with one volume of animal charcoal. The clear fluid which passed through the filter was found to have lost half its rotating power.

It follows from these experiments, that a large excess of animal charcoal retains much sugar, and that the more charcoal used the less sugar passes through the filter; but all the sugar that is kept back can be washed out with boiling water.

It was desirable to know how much charcoal could be used without perceptible loss of sugar. 50 c. c. of a pure solution of sugar gave 27° of rotation. It was mixed with between 55 and 60 grains of charcoal shaken and filtered, and then gave 26°.

25 c. c. of a nearly colourless diabetic urine were diluted to 55 c. c.; the rotation was then between 15° and 16°. 50 c. c. of the same urine, shaken with 60 grs of charcoal, gave a colourless solution which rotated between 31° and 32°.

Some experiments were made with wood-charcoal to see its effects on sugar and on the colouring matter of the urine.

The wood-charcoal was finely powdered, but not fresh burnt.

One volume of pure sugar solution was shaken with half a volume of wood charcoal, and left to stand for some time. The solution gave 19° of rotation both before and after treatment. The same charcoal was shaken with urine, the fluid which filtered through was nearly as dark coloured as at first.

These experiments were repeated with fresh burnt wood charcoal. The solution of sugar was not affected. The colouring matter of the urine was partly removed, but the whole of the colour could not thus be taken away.

On the action of basic and neutral acetate of lead on solutions of sugar.

Dr. E. Robiquet, in his instructions for using his diabetometer, says that 25 c. c. of diabetic urine are to be mixed with 1 c. c. of extract of lead and 1 c. c. of liquid ammonia. The whole is to be shaken for some minutes to give a deposit. If the clear liquid is not completely decolorised, the same quantity of lead and ammonia is again to be added. If the decoloration is then complete, the amount of sugar may be determined by the amount of rotation observed.

25 c. c. of sugar-solution were diluted to 50 c. c. with water. Two experiments gave 21° and 22° of rotation. The same quantity of the same solution was mixed with 20 c. c. of neutral acetate of lead and 2 c. c. of ammonia, and the whole was diluted to 50 c. c. after filtration the rotation was found to be, in the first experiment, between 18° and 19°, and in the second experiment 18°. 25 c. c. of sugar-solution diluted to 50 c. c. gave between 9° and 10° of rotation. 25 c. c. of same solution with 2 c. c. of acetate of lead and 2 c. c. of liquid ammonia, and the whole diluted to 50 c. c. gave only 7° of rotation.

By using three times as much solution of lead and ammonia, the rotation was only 4° to 5°.

On the action of basic acetate of lead alone.

50 c. c. of urine were mixed with a solution of sugar which gave 9° of rotation; after precipitation by basic acetate of lead it was found to have lost 3° of rotation. A second experiment gave the same result.

A urine which gave between 8° and 9° of rotation gave from 5° to 6° after precipitation. The lead-precipitate was washed with hot water on the filter and boiled with hot water, but the sugar could not be removed.

In order to compare the action of basic and neutral acetate of lead on saccharine urine and on solutions of sugar in water alone, and in water with common salt and water and phosphate of soda, the following experiments were made.

A pure sugar-solution gave 10° to 11° of rotation; when mixed with common salt and precipitated by basic acetate of lead, it gave the same rotation; when more common salt was used, no difference was observed.

A solution of sugar which gave 9° of rotation was mixed with solution of common salt and urate of soda, and precipitated by basic acetate of lead; the rotation was then found to be between 7° and 8°. When the quantity of salt and urate of soda was less, the rotation was 8°.

A solution of sugar giving between 10° and 11° of rotation was mixed with much phosphate of soda, and then precipitated by basic acetate of lead: it then gave 8° to 9°.

A solution of sugar which gave 5°, after being mixed with much phosphate of soda and precipitated by basic acetate of lead, gave from 3° to 4° of rotation.

A solution giving 10° to 11°, mixed with a little urate of soda, and then precipitated by basic acetate of lead, gave 10° of rotation.

When neutral acetate of lead was used instead of basic acetate, very different results were obtained.

A pure sugar-solution gave 10° to 11° of rotation. When the solution was mixed with common salt, phosphate, and urate of soda, it gave, after precipitation, 10° to 11° of rotation, and the rotation was unchanged when a greater amount of these salts was added before precipitation.

1st. These experiments confirm the fact that a pure solution of sugar is not precipitated either by basic or by neutral acetate of lead, but that sugar is precipitated by neutral acetate of lead and ammonia.

2nd. Basic acetate of lead when added to saccharine urine causes the precipitation of some sugar. The urates and phosphates in the urine cause this precipitation of sugar, and not the chloride of sodium; for when a solution of sugar in water is mixed with chloride of sodium, basic acetate of lead causes no precipitation of the sugar. But when urate or phosphate of soda also is present, then some sugar is precipitated.

3rd. When neutral acetate of lead is added to solutions of

sugar containing chloride of sodium, phosphate, and urate of soda, no precipitate of sugar occurs.

On the action of Chlorine.

Into a solution of sugar in water containing 6 grains of sugar to 20 c.c. of water, chlorine gas free from hydrochloric acid was passed for half an hour. It was left for twenty-four hours, and then hardly any difference of rotation was observed.

Into a solution of the same strength, chlorine was passed for an hour and a half, and it then was left for twenty-four hours; the loss was less than a grain of sugar.

325 c. c. of urine were mixed with 3 grains of sugar and to 20 c. c. The concentrated fluid was heated for twenty minutes with chlorine. It had then a yellowish urine colour, which could not be removed by further exposure to chlorine, and the subsequent use of animal charcoal did not give a solution which could be examined by the saccharimeter.

Pettenkofer's Test.

The fluid in which sugar is suspected is decolorised as far as possible, and then mixed with a few drops of a concentrated solution of glychocholic acid in soda or cholalic acid. Three or four drops of concentrated sulphuric acid are then added, and the whole gently heated without boiling. If sugar is present, a purple colour is seen at the edge of the watch glass: this is more evident on a white ground.

A standard solution of grape-sugar was made, 1 c. c. containing 0·005 sugar, and 5 drops of the solution = 1 milligramme, and was mixed with cholic acid and concentrated sulphuric acid: intense purple blue immediately formed.

One drop of this solution = 0·0002 of sugar, mixed with cholic and sulphuric acid gave, after a few minutes, a slight purple red and ultimately a tinge of blue.

5 drops of diabetic urine containing 7 grs. of sugar to the ounce of urine were mixed with cholic acid, and a strong sulphuric acid. After a few minutes, a slight purple red appeared which ultimately became bluish.

One drop of this diabetic urine without decoloration gave a purple red. Cholalic acid was found to have the same reaction as cholic acid.

Trommer's Test.

Among the different ways of employing this test, that recommended by Lehmann was found to be best. By it, $\frac{1}{20}$ per cent. (or 0·24 gr. to 1 oz.) of sugar added to the urine was easily detected by deposit of suboxide of copper.

2 or 3 c. c. of urine are mixed with a few drops of potassa and filtered, and then an equal quantity of strong potassa is added, with about 3 drops of a solution of sulphate of copper; the whole is well shaken, and the clear liquid poured off from the hydrated oxide of copper which has not dissolved. If the blue solution when heated (long boiling is quite unnecessary) becomes colourless without depositing suboxide of copper, then two drops more of the sulphate of copper should be added, and the experiment repeated. A separation of suboxide is often thus obtained, provided the boiling has not been continued so long at the first heating as to decompose the sugar.

Pure grape-sugar in water gives the well-known red suboxide. In urine, the suboxide is bright yellow or dirty yellow. When 0·01 gramme of grape-sugar dissolved in water was precipitated by acetate of lead and ammonia, and the precipitate treated with a little oxalic acid solution, the sugar solution gave a dirty yellowish reduction with standard copper-solution.

A solution of grape-sugar was mixed in different proportions with a solution of chloride of ammonium. The separation of the suboxide of copper was stopped, when the solution containing $\frac{1}{200}$ of a grain of sugar also contained 1 grain of chloride of ammonium. In some experiments, the suboxide of copper was deposited whilst ammonia in quantity was being given off.

The same series of experiments were made with urea and grape-sugar: the urea hindered the separation of the suboxide, when a solution containing $\frac{4}{1000}$ of a grain of sugar contained also 1 grain of urea.

Brücke's Processes.

Professor Brücke has published two processes for detecting small quantities of sugar in urine.

In the first or alcohol process, he advises that the urine should be mixed with four times its bulk of absolute alcohol. An alcoholic solution of potassa is then to be added, and the fluid is left for twelve hours to deposit potassa-sugar. The alcohol is then to be poured off, and the deposited matter dissolved in water and tested by reduction and other tests.

According to the experiments described in the early part of this paper, when small quantities of sugar exist in solution, an alcohol of 80 per cent. will only give from one-third to a scarcely perceptible quantity of the sugar which existed in a solution. If a very small quantity of sugar was present, this percentage of alcohol would therefore fail to detect it. With absolute alcohol by this process, the whole of the sugar is precipitated, but if the percentage of alcohol falls below 80, little or no sugar will be obtained. An alcohol of 90 per cent. gives only half the sugar that is present; and hence this method of Professor Brücke is very imperfect and very costly; even with methylated spirit.

In his second process, he recommends that the urine should be precipitated with neutral acetate of lead and then with basic acetate of lead, and after filtering off the precipitate, ammonia should be added; in this last precipitate the chief part of the sugar present will be found. What is the amount of sugar which, when added to the urine, can be detected by this process?

145 c. c. of fresh morning urine were treated with acetate of lead, basic acetate of lead, and then ammonia. 5 c. c. of a standard solution of sugar containing 0·025 gramme = $\frac{1}{3}$ of a grain nearly of sugar, was added before precipitation. The solution was almost free from colouring matter after precipitation by basic acetate of lead and quite free when precipitated by ammonia.

On adding 20 drops of a standard solution of copper to the potass solution of the subacetate of lead, no red suboxide of copper formed, but a dirty suboxide of copper fell. The solution of the ammonia precipitate in oxalic acid gave only a trace of red oxide, but plenty of dirty coloured suboxide. In the cold after 24 hours the same reduction occurred.

200 c. c. of fresh morning urine were treated as in the last experiment, but only 0·012 gramme = $\frac{1}{6}$ of a grain of sugar was added. The oxalic acid solution of the ammonia precipitate gave with 10 drops of copper solution a slight reduction, a dirty yellowish precipitate was obtained on boiling. The potash solution of the subacetate of lead precipitate destroyed the blue colour of the sulphate of copper solution but gave no precipitate.

200 c. c. of fresh mid-day urine were treated as before, 0·05 gramme = $\frac{3}{4}$ of a grain of sugar was added. The three precipitates were examined as well as the solution filtered from the ammonia precipitate.

1. The acetate of lead precipitate gave no reduction.

2. The basic acetate of lead precipitate was dissolved in potassa and gave no reduction; the blue colour of the copper solution disappeared however.

3. The precipitate with ammonia was treated with solution of oxalic acid, and on the addition of 4 c. c. of standard-copper solution a good reduction was obtained of a yellowish colour.

4. The clear liquid from the ammonia precipitate gave no reaction of sugar.

200 c. c. of fresh urine were treated as before; only ·01 gramme = $\frac{1}{7}$ of grain of sugar was added. The oxalic acid solution contained as usual the whole of the sugar. 2 c. c. of copper solution gave a dirty yellowish precipitate.

When the same quantity of grape-sugar was added to distilled water and treated in the same way, the oxalic-acid solution tested by the standard copper solution gave the same dirty-yellow precipitate.

To 700 c. c. of urine a known quantity of solution of grape-sugar giving 12° of rotation of the saccharimeter was added. The urine was treated as before. The ammonia precipitate decomposed by oxalic acid, contained sufficient sugar when diluted to the known quantity to give a rotation of 8°. Instead of decomposing the ammonia precipitate by oxalic acid, sulphuretted hydrogen was used, and if requisite this was twice repeated; the filtrate is then almost colourless, even when 5000 c. c. of urine have been used for an experiment.

To 1300 c. c. of urine a known quantity of a solution of grape sugar was added, giving 13° of rotation. In the ammoniacal precipitate enough sugar was found to give between 7° and 8° of rotation.

To 1137 c. c. of urine a known quantity of sugar solution was added, giving 11° to 12° of rotation in the ammoniacal precipitate; enough sugar was found to give, with the same quantity of water, 8° to 9° of rotation. This experiment when repeated gave in the ammoniacal precipitate 8° of rotation.

Hence by Brücke's lead process, when $\frac{3}{4}$, $\frac{1}{3}$, $\frac{1}{6}$, $\frac{1}{7}$ of a grain of grape-sugar are added to about 200 c. c. of urine, decided evidence of sugar was found in the ammonia precipitate. And by the quantitative experiments it appears that about two-thirds of all the sugar added can be recovered by this process.

The results of these experiments on the detection of sugar when added to the urine may be thus summed up:

1. Lehmann's process for detecting sugar in the urine cannot be employed when small quantities of sugar are present in large quantities of urine; by evaporation and decoloration, all the sugar is lost.

2. The process of fermentation is stopped by the residue of the urine, by much urea and by oxalate of urea still more decidedly. Half a grain of sugar in water can be detected by the alcohol produced, and may be determined approximatively by the carbonic acid given off; but in concentrated urine much larger quantities will be entirely overlooked.

3. In decolorising the urine for use in the saccharimeter, sugar is always lost; animal charcoal retains sugar in proportion to the amount of charcoal used. When the urine is decolorised by basic acetate of lead and ammonia, two-thirds of the sugar may be lost.

4. Pettenkofer's test for sugar is the most delicate test known; $\frac{2}{3}$ of a milligramme in distilled water, can be detected by it, and a little colouring matter of the urine does not hinder the reaction. If much colouring matter is present, it must be removed.

5. Trommer's test is capable of discovering $\frac{1}{200}$ per cent. of sugar in the urine, but when very small quantities of sugar are in solution with hydrochlorate of ammonia or urea, the reduction of the oxide of copper is not perceived; $\frac{1}{200}$ of a grain of sugar with 1 grain of hydrochlorate of ammonia, in water, gives no reduction; and $\frac{4}{1000}$ of a grain of sugar with 1 grain of urea stopped reduction.

6. Brücke's processes.

In the alcohol process if 80 per cent. alcohol is used, only $\frac{1}{4}$ or less of the sugar is obtained: and even by 90 per cent. alcohol one-half is lost. The necessity for so much absolute alcohol as will give with the urine a mixture of 90 per cent., makes the process nearly useless.

By Brücke's lead process, the best results have been obtained; $\frac{1}{7}$ of a grain of sugar in 200 c. c. of urine could be detected, and $\frac{2}{3}$ of all the sugar added was recovered. Moreover, when sulphuretted hydrogen is used to decompose the ammonia precipitate, the sugar is obtained in a state fit for fermentation, and free from colour, so that the saccharimeter can be employed.

PART 2.—*On the detection of sugar naturally present in healthy urine.*

The presence or absence of sugar in healthy urine is not only of great interest in relation to the true comprehension of the nature of diabetes, but it is also of importance in respect to

our knowledge of the chemical changes which occur in the body in health.

If sugar exists in the urine in health, as Brücke maintains, then diabetes must be considered as an exaggeration of a healthy state, and not as a distinct and peculiar condition of the system; and it will be necessary to admit, that in health and in diabetes, the same chemical changes take place in the system, but that the greater amount of change in the one case constitutes health, and the lesser amount in the other case is called diabetes.

Professor Brücke deserves all credit for the accuracy of his observations, and for the clearness of his statements; and though I failed by the alcohol process to satisfy myself of the truth of the results which he obtained by that method, yet, by his lead process, I have fully convinced myself, after a lengthened investigation, which only the perseverance of Dr. Ulrich could have carried out, that there is sugar in healthy urine; and that, in addition to the proofs of its presence mentioned by Professor Brücke, it may be detected and measured by the saccharimeter; and that by fermentation, alcohol in recognisable quantity may be obtained.

On the detection of sugar in healthy urine, by Brücke's alcohol process.

140 c. c. and 200 c. c. of fresh urine were mixed with four times their bulk of alcohol sp. gr. 808 (or 95 per cent.), a solution of potassa in the same strength of alcohol was added, and the fluid was left for twelve hours to allow the potass-sugar to deposit itself. The liquid was then poured off; the deposited matter was dissolved in water, and tested by Trommer's test, Böttger's test, and a solution of potassa alone. No conclusive result was obtained.

Three other experiments were then made with 1,000 c. c. of urine and alcohol of the same strength. The copper test gave a decided reduction; the potassa alone hardly deepened in colour. The bismuth test was not conclusive; on testing the deposit from the alcohol for uric acid, it was found to be present in considerable quantity. In the Medico-Chirurgical Transactions vol. 26, p. 215 (1843), I have shown that uric acid reduces the oxide of copper in Trommer's test. Hence the reduction obtained in these three experiments was no proof of sugar being present, and I determined to try whether, by using large quantities of urine, sufficient sugar

could be precipitated to admit of the determination of its presence and amount by the saccharimeter.

3,000 c. c. of urine (about 5 pints) were added to 12,200 c. c. of alcohol (above 21 pints). This quantity was divided into three portions; potassa dissolved in alcohol was added to each portion before it was set aside to deposit the sugar-potassa. The precipitate was dissolved in water, neutralised with oxalic acid, filtered through animal charcoal, and concentrated on a water-bath to about one ounce (30 c. c.). The clear fluid was examined by the saccharimeter, but no trace of rotation could be observed.

In another experiment, 1,000 c. c. of fresh urine were mixed with 4,000 c. c. of alcohol, and treated in the same way; but no trace of rotation was observed.

Another series of three portions of 1,000 c.c. of urine each, was mixed with 12,000 c.c. of alcohol, sp. gr. 808. The smallest quantity of water was used for dissolving the potassa sugar; a very small quantity of animal charcoal was used for decolorising the solution, which was examined by the saccharimeter; but no rotation could be seen.

Failing thus to detect sugar by the saccharimeter, I used Pettenkoffer's test on fresh portions of urine.

200 c. c. were mixed with alcohol and treated with potassa. A very small quantity of cholalic and of cholic acids was dissolved in concentrated sulphuric acid, and some of the fluid thought to contain sugar was added, but no purple tint in either case was produced. The test failed to find sugar, though the same solutions detected 2 milligrammes of sugar (·03 of a grain) dissolved in 10 drops of water, and one drop of diabetic urine, containing ·05 grain of sugar, gave a fine purple colour. As it was possible that by using a larger quantity of urine, some trace of sugar might be found, 3,000 c. c. of urine were precipitated by alcohol. The deposit was dissolved in a very small quantity of water and decolorized by animal charcoal, but no decided evidence of sugar was obtained.

At the time when these experiments were made with the alcohol process, I did not know how little sugar was precipitated by potassa from 80 per cent. alcohol. As this process failed, the lead process was tried.

On the detection of sugar in healthy urine by Brücke's lead process.

200 c. c. of healthy morning urine, passed by A, were treated

with lead and ammonia; the basic acetate of lead precipitate was dissolved in a small quantity of potassa, and the solution was tested with Fehling's standard-copper solution; no reduction was obtained, but the blue colour of the copper solution disappeared, and the liquid became of a light amber colour, but not a trace of suboxide of copper was seen. The ammonia precipitate was treated in the cold with oxalic acid, and the filtrate was examined for sugar by the copper solution, but no reduction occurred.

200 c. c. of mid-day urine (A) were treated exactly in the same way, and with exactly the same results.

500 c. c. of healthy urine (A) treated in the same way with oxalic acid gave no decided evidence of sugar by reduction, or by Pettenkoffer's test. This experiment repeated with another quantity of urine gave the same result. When in another experiment, the ammoniacal precipitate was decomposed by sulphuretted hydrogen, decided evidence of reduction was obtained.

1,000 c. c. of same urine (A) treated with oxalic acid instead of sulphuretted hydrogen, gave a reduction which was not sufficiently distinct. When this experiment was repeated and sulphuretted hydrogen employed, a distinct reduction of the oxide of copper took place, and Pettenkofer's test also showed that sugar was there. This last experiment was three times repeated, and reduction always occurred.

1,000 c. c. of the urine of another healthy man, (B) was treated in the same way by lead and sulphuretted hydrogen; the clear filtrate was evaporated and it reduced the copper solution readily.

2,000 c. c. of the urine (A) gave a very plentiful reduction. The experiment was repeated; the filtrate from the sulphide of lead was evaporated to dryness, and extracted with absolute alcohol; potassa-alcohol was then added; and a deposit formed on standing, which gave a good reduction.

It was requisite to prove that the reduction was caused by sugar. For this purpose, larger quantities of urine were treated in the same way, and the ammonia precipitate was tested for sugar by the saccharimeter and by fermentation.

5,000 c. c. of urine (A) were examined; the filtrate from the sulphide of lead was evaporated in vacuo; and the residue dissolved in a little water was decolorised by a little animal charcoal; half the solution examined by the saccharimeter gave 4° of rotation. In two other experiments with different quantities of the urine (A), 2° and 3° degrees of rotation were observed.

5,000 c. c. of urine (B) treated in the same way gave 5° of rotation.

1,100 c. c. of the urine of a third person (C) treated in the same way, gave $1\frac{1}{2}$° of rotation; and by the reduction-test sugar was easily found.

In two experiments with 10,000 c. c. of urine (A) treated with oxalic acid, the decoloration could not be made so as to allow of the use of the saccharimeter; but the reduction-test and Pettenkofer's test gave full evidence of sugar.

Hence, in those different healthy persons, and in six different experiments, the rotation showed that sugar was present in the urine.

On the amount of rotation observed in healthy urine.

Two more experiments were made with the urine of (A) and (B), to determine the greatest amount of rotation in health.

5,000 c. c. of the urine (A) were treated as before with sulphuretted hydrogen. The whole of the fluid was used in the saccahrimeter, and the rotation was between 7° and 8°.

5,000 c. c. of the urine (B), when all the fluid was used, gave from 10° to 11° of rotation.

On the proof of the presence of sugar in healthy urine by fermentation.

10,000 c. c. (10 litres) of the urine (A) were treated with sulphuretted hydrogen. The filtrate was evaporated, and half was fermented in two portions.

	1st Portion.	2nd Portion.
Yeast employed . .	31·22 grs.	29·30 grs.
Total carbonic acid given off	0·80 gr.	0·86 gr.
Carbonic acid from yeast .	0·43 gr.	0·41 gr.
Carbonic acid from sugar .	0·37 gr.	0·45 gr.

Hence total carbonic acid from sugar = 0·82 gr. = 1·68 gr. sugar; so that the total quantity thus obtained from 10 litres was 3·36 grs. sugar.

14,000 c. c. of urine (A), after the filtrate from the sulphide of lead was evaporated to dryness, had the sugar precipitated from

absolute alcohol by potassa. The precipitate was dissolved in water, neutralised by hydrochloric acid and evaporated; the residue extracted by strong alcohol; the alcoholic solution again evaporated to dryness; and the residue again dissolved in water, mixed with yeast which had been well washed, and kept at a temperature between 25° and 30° C.: (77° and 86°) F. in half an hour it began to ferment.

Yeast employed in sugar-solution . .	34·2 grs.
Carbonic acid given off	1·8 gr.
Yeast without sugar-solution . .	109·6 grs.
Carbonic acid given off	0·3 gr.

Therefore carbonic acid in 34·2 grs. of yeast may be neglected.

The yeast alone mixed with water and distilled, gave a scarcely perceptible trace of reduction when the distillate was added to a solution of chromic acid, whereas the other yeast-mixture distilled gave a fluid which showed plentiful reduction, proving that alcohol was present after fermentation.

The total carbonic acid given off = 1·8 gr.; this corresponds to 3·7 grs. sugar obtained from 14 litres of healthy urine.

An undetermined quantity of the different fluids which had been used in the experiments with the saccharimeter was evaporated; the residue extracted with strong alcohol; the alcoholic solution evaporated; the residue again redissolved in strong alcohol; and the solution was evaporated to dryness, dissolved in water, and mixed with slightly washed yeast.

Yeast used	44·6 grs.
Carbonic acid . . .	2·4 grs.

Yeast without sugar-solution 100·3 grs.; carbonic acid 0·7 gr. Hence 44·6 yeast give 0·3 gr. carbonic acid.

Hence carbonic acid from sugar = 2·4 grs. − 0·3 = 2·1 grs. = 4·3 grs. sugar.

The fluid distilled from yeast alone reduced chromic acid; the fluid which was distilled from the other apparatus reduced the chromic acid much more decidedly.

On the determination of the amount of sugar present in healthy urine.

Although the saccharimeter and fermentation afford the most

satisfactory proof of the presence of sugar, yet the quantitative determination by either of these methods is not so accurate or so useful as by means of Fehling's standard-copper solution; because the processes for decolorising and for separating the sugar from the urine cause a great loss, which does not occur when the amount of sugar is determined by a standard solution, because then a smaller quantity of urine will give conclusive results.

1,000 c. c. (1 litre) of urine (A) was treated as before; the filtrate from the sulphide of lead was evaporated on a water-bath, decolorised with a little animal charcoal, and then tested by Fehling's solution.

The volume of fluid was 29 c. c.; of this 15 c. c. were required to reduce 10 c. c. of Fehling's solution. Hence the 29 c. c. contained 0·09 gramme sugar = 1·4 grain, as by the process one-third of the sugar present is lost. The litre contained about 2·2 grs. of sugar.

In another litre (A) the volume of fluid was 18 c. c.; of this 12 c. c. reduced 10 c. c. of Fehling's solution. Hence 18 c. c. contained 0·07 gramme sugar = 1·08 gr. Adding one-third to this, we find that the litre contained about 1·5 grains.

In another litre (A) the volume of fluid was 18 c. c.; of this 13 c. c. reduced 10 c. c. of Fehling's solution. Hence the 18 c. c. contain 0·06 gramme sugar = ·924 gr. One-third added = 1·38 gr. to litre urine.

In another litre (A), the volume of fluid was 18 c. c.; of this 11 c. c. reduced 10 c. c. of Fehling's solution. Hence the 18 c. c. contain 0·08 gramme sugar = 1·23 gr. One-third added = 1·8 gr. sugar to litre urine.

A litre of urine (B) was treated in the same way. The volume of fluid was 18 c. c.; of this 7 c. c. reduced 10 c. c. of Fehling's solution. Hence the 18 c. c. contain 0·13 gramme sugar = 2·0 grs. One third added = 3·3 grs. sugar to litre urine.

In another litre (B), the volume of fluid was 26·6 c. c.; of this 12·6 c. c. reduced 10 c. c. of standard solution. Hence the 26·6 contain 0·1 gramme sugar = 1·5 gr. One-third added = 2·3 grs. sugar to litre urine. By the saccharimeter also evidence of rotation was obtained.

Hence,	1st Exp.	2nd Exp.	3rd Exp.	4th Exp.
1 litre (A) urine contained	2·2 grs.	1·54	1·38	1·8 gr. sugar.
1 litre (B) urine contained	3·0 grs.	2·3 grs. sugar.		

From these experiments on healthy urine it follows:—

1. That by the alcoholic process, when 140 c. c. to 3,000 c. c. of urine were employed, no result was obtained by the reduction test, by Pettenkofer's test, or by the saccharimeter, chiefly because the percentage of alcohol was not great enough.

2. That by the lead process, most conclusive results were obtained, qualitatively and quantitatively, by the reduction test, by fermentation, and by the saccharimeter.

By the reduction test, qualitatively, 200 c. c. of urine gave no evidence; 500 c. c. failed, and 500 c. c. gave proof of sugar; 1,000 c. c. gave very evident sugar; 3,000 c. c. gave much more proof. By this test, quantitatively 1,000 c. c. of one man gave 2·2, 1·5, 1·4, and 1·8 grs. of sugar. The same quantity of urine in another man gave 3·0 and 2·3 grs. of sugar in one litre.

By the saccharimeter, quantitatively, 5,000 c. c. of urine, partly used, gave 2°, 3°, and 4° of rotation. In another man, 5° were observed. In a third man, a smaller quantity of urine gave 1° to 2° of rotation.

By this test quantitatively, 5,000 c. c. of one man, all used, gave 7° to 8° of rotation. The same quantity from another man, gave 10° to 11°.

By fermentation, qualitatively, an unknown quantity fermented, gave distinct evidence of alcohol and 2·1 grs. of carbonic acid.

Quantitatively, 10,000 c. c. of urine were used, and ultimately, 1·64 gr. of carbonic acid was obtained.

14,000 c. c. of the same urine gave most positive proof of alcohol and 1·8 gr. of carbonic acid.

These experiments, therefore, fully confirm Professor Brücke's statement, that sugar exists in healthy urine. By obtaining alcohol from the fermented fluid, and by never failing to find rotation when the saccharimeter was used, provided sufficient urine had been taken for the experiments, I have added to the evidence given in his original paper.